Date: 2025 -02

Supplementary information on EN 18032 (informative)

Foodstuff — Quick Method for the Analysis of Multiple Highly Polar Pesticides and their Metabolites in Foodstuff Involving Extraction with Acidified Methanol and Measurement by LC- or IC-MS/MS (QuPPe-Method) - Supplementary information on the method

Lebensmittel — Schnellmethode zur Bestimmung mehrerer hochpolarer Pestizide und ihrer Metaboliten in Lebensmitteln nach Extraktion mit angesäuertem Methanol und Messung mittels LC- oder IC-MS/MS (QuPPe-Methode) – Ergänzende Informationen zur Methode

Produit alimentaire — Méthode rapide pour l'analyse de plusieurs pesticides hautement polaires et de leurs métabolites dans les aliments impliquant une extraction avec du méthanol acidifié et une mesure par LC- ou IC-MS/MS (QuPPe-Method) – Informations complémentaires sur la méthode

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Supplement A (informative):

Schemes showing the method at a glance

SA.1 QuPPe Method at a glance (for most fruits and vegetables)

WEIGH sample homogenate into 50 mL centrifuge tube

Fresh fruits and vegetables (with high water content): 10 g \pm 0.1 g (E1) Previously re-hydrated dry fruit: e.g. 13.5 g \pm 0.1 g (containing 5 g sample) (E3) Dry commodities (e.g. herbs): 2 g \pm 0.02 g (E5a)

E1: ADJUST WATER CONTENT of sample to 10 mL

(Mandatory for matrices w. <80% water. If no IL-IS used manadatory for ALL matrices) e.g. +10 mL of water to 2g of dried mint (E5);

+2 mL water to 10 g potato (E2); + 3.5 mL water to 10 g garlic (E2)

Add 100 µL isotopically-labeled internal standard (IL-IS) mix

E1/E2/E3/E5a: ADD EXTRACTION SOLVENT (10 mL methanol containing 1 % formic acid)

E1/E2/E3/E5a: SHAKE thoroughly for 1 min to 15 min for dry commodities

C1: Preferably FREEZE-OUT extract until completely frozen

e.g. >90 min at -18°C or ca. 30 min at -80°C

CENTRIFUGE (5 min at >3,000 g but preferably >10,000 g);

preferably cryogenic centrifugation (e.g. at -10 °C)

(if centrifuge is not refrigerated, swiftly proceed with centrifugation and the following step to avoid redissolvation of matrix)

C2: dSPE to Remove Lipids for High Oil Content samples (e.g. avocado): (this step may be skipped if sample was centrifuged frozen at \leq -10 °C and \geq 20 min)

TRANSFER 4 mL raw extract into a tube containing 200 mg C_{18} -sorbent, SHAKE for 1 min and CENTRIFUGE (>3,000 g for 5 min)

WITHDRAW SUPERNATANT AND FILTER it into a plastic autosampler vial

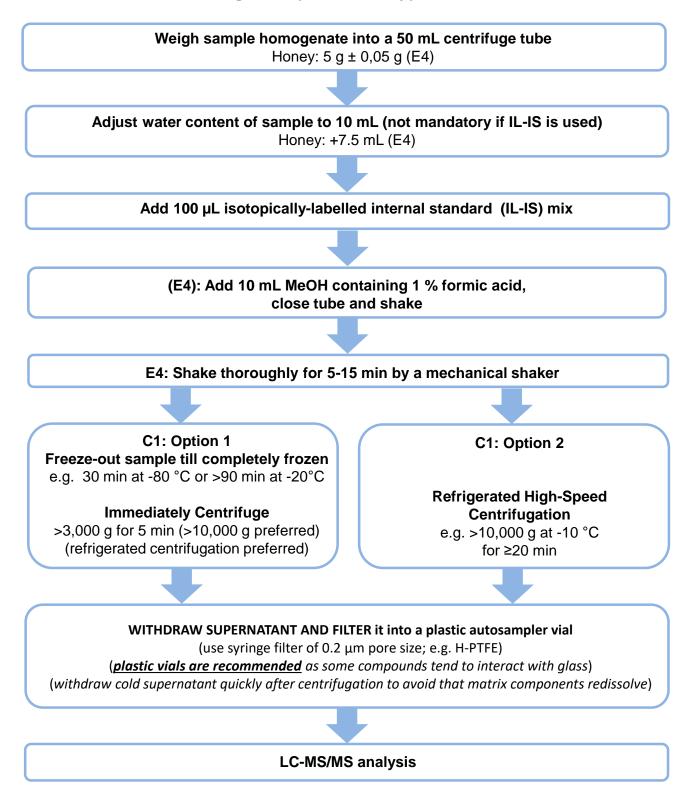
(use syringe filter of 0.2 µm pore size; e.g. H-PTFE)

(*plastic vials are recommended* as some compounds tend to interact with glass)

(withdraw cold supernatant quickly after centrifugation to avoid that matrix components redissolve)

LC-MS/MS and IC-MS/MS analysis

SA.2 QuPPe Method at a glance (for E4 honey)



SA.3 QuPPe Method at a glance (for E5b cereals, pulses, nuts and oily seeds)

E5b: Weigh 5 ± 0.05 g of sample homogenate into a 50 mL centrifuge tube E5b: Add 9 mL of water Add 100 µL isotopically-labelled internal standard (IL-IS) mix E5b: Add 10 mL MeOH containing 1 % formic acid + extra 100 μL formic acid close tube and shake E5b: Add 1 mL 10% aqueous EDTA solution E5b: Shake thoroughly for 15 min by a mechanical shaker C1: Option 1 C1: Option 2 Freeze-out sample e.g. 30 min at -80 °C or >90 min at -20 °C **Refrigerated High-Speed Centrifugation Immediately Centrifuge** e.g. >10,000 g at -10 °C for ≥20 min >3,000 g for 5 min (>10,000 g preferred) (refrigerated centrifugation preferred) C2+C3: Removal of proteins and lipids Transfer 2 mL of raw extract into a tube containing ... a) C2+C3: Oily Seeds, Nuts: 2 mL acetonitrile and 100 mg C₁₈-sorbent b) C3: Pulses and Cereals: 2 mL acetonitrile Shake for 1 min and centrifuge at >3,000 g for 5 min C4: Filter aliquot of supernatant Centrifugation assisted ultrafiltration through a 5 or 10 kDa cut-off filter (e.g. polyethersulfone membrane) LC-MS/MS and IC-MS/MS analysis

SA.4 QuPPe Method at a glance (for E6a liver, milk, kidney, egg and muscle)

Weigh sample homogenate into a 50 mL centrifuge tube

Milk, Egg, Kidney, Muscle and Liver: 10 g ± 0,1 g (E6a)



Liver: +3 mL; Muscle +2.5 mL; Kidney +2 mL, Egg +2.5 mL, Whole fat milk: +1.5 mL (E6a); skimmed milk: no addition



Add 100 µL isotopically-labelled internal standard (IL-IS) mix

Liver, Milk, Kidney, Muscle, Egg (E6a):
Add 10 mL MeOH containing 1 % formic acid,
close tube and shake



E6a: Shake thoroughly for 1-15 min by a mechanical shaker



C1: Option 1

Freeze-out sample till completely frozen

e.g. 30 min at -80 °C or >90 min at -20 °C



>3,000 g for 5 min (>10,000 g preferred) (refrigerated centrifugation preferred)



Refrigerated High-Speed Centrifugation

e.g. >10,000 g at -10 °C for ≥20 min



C2+C3: dSPE and dilution with ACN for removal of lipids and protein precipitation

Transfer 2 mL of raw extract into a tube containing 100 mg C_{18} -sorbent and 2 mL ACN, Shake for 1 min and centrifuge at >3,000 g for 5 min



C4: Filter aliquot of supernatant

Centrifugation assisted ultrafiltration through a 10 kDa cut-off filter (e.g. polyethersulfone membrane)



LC-MS/MS analysis

SA.5 QuPPe Method at a glance (for E6b liver, milk, kidney, egg and muscle)

Weigh sample homogenate into a 50 mL centrifuge tube

Milk, Egg, Kidney, Muscle and Liver: 10 g \pm 0,1 g (E6b)

Adjust water content of sample to 10 mL (not mandatory if IL-IS is used)

Liver: +2 mL; Muscle +1.5 mL; Kidney +1 mL, Egg +1.5 mL, Whole fat milk: +0.5 mL (E6b); skimmed milk: no addition

Add 100 µL isotopically-labelled internal standard (IL-IS) mix

Liver, Milk, Kidney, Muscle, Egg (E6b):
Add 10 mL MeOH containing 1 % formic acid + extra 100 μL formic acid,
close tube and shake

E6b: Add 1 mL 10% aqueous EDTA solution

E6b: Shake thoroughly for 1-15 min by a mechanical shaker

C1: Option 1 Freeze-out sample till completely frozen

e.g. 30 min at -80 $^{\circ}$ C or >90 min at -20 $^{\circ}$ C

Immediately Centrifuge

>3,000 g for 5 min (>10,000 g preferred) (refrigerated centrifugation preferred)

C1: Option 2

Refrigerated High-Speed Centrifugation

e.g. >10,000 g at -10 °C for ≥20 min

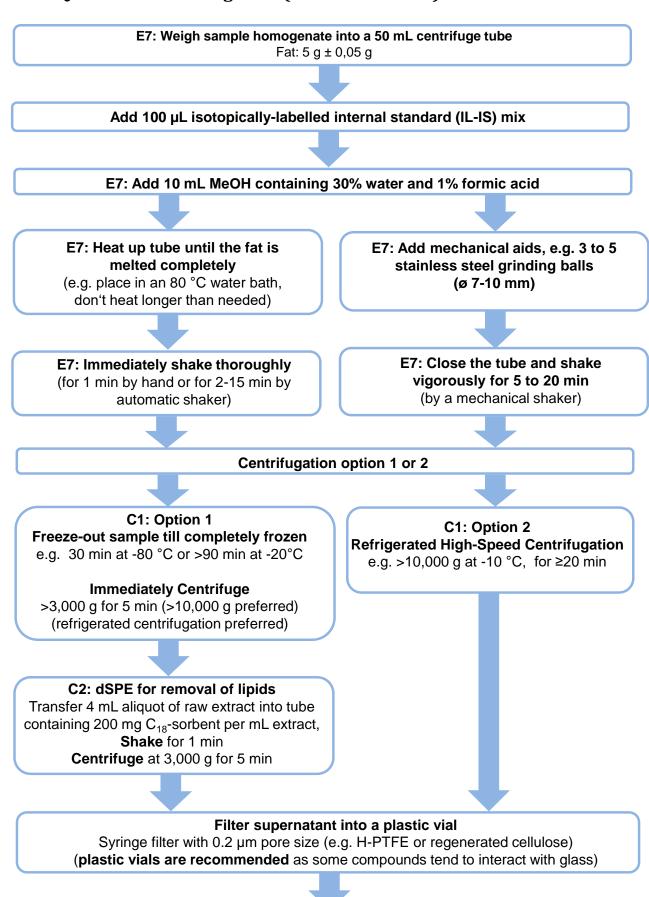
C2+C3: dSPE and dilution with ACN for removal of lipids and protein precipitation Transfer 2 mL of raw extract into a tube containing 100 mg C₁₈-sorbent and 2 mL ACN, Shake for 1 min and centrifuge at >3,000 g for 5 min

C4: Filter aliquot of supernatant

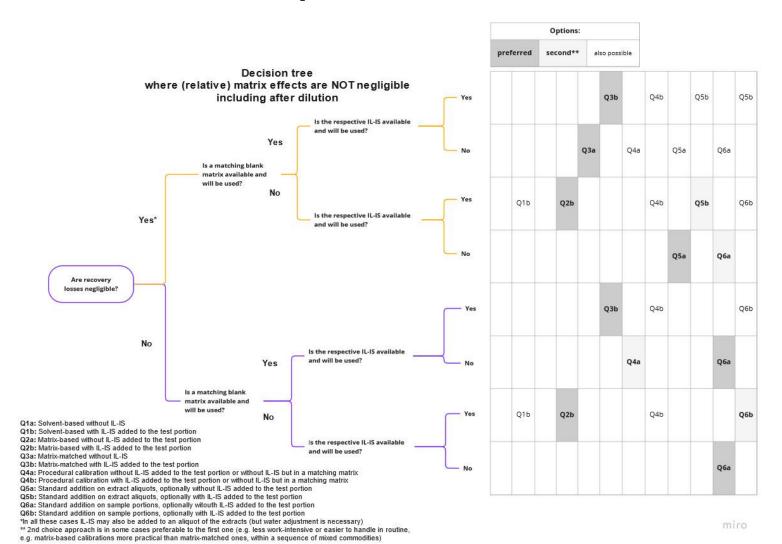
Centrifugation assisted ultrafiltration through a 10 kDa cut-off filter (e.g. polyethersulfone membrane)

LC-MS/MS analysis

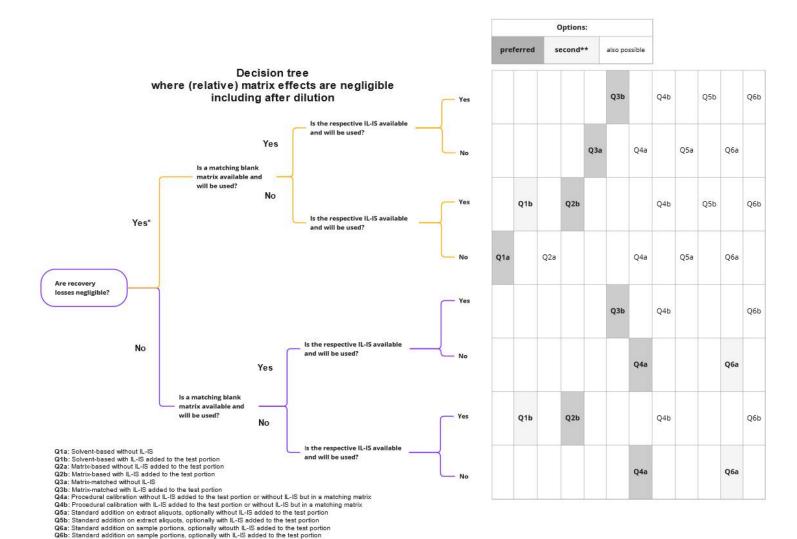
SA.6 QuPPe Method at a glance (for E7 animal fat)



SA.7 Decision trees on the use of quantification modules



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"In all these cases IL-IS may also be added to an aliquot of the extracts (but water adjustment is necessary)

" 2nd choice approach is in some cases preferable to the first one (e.g. less work-intensive or easier to handle in routine,
e.g. matrix-based calibrations more practical than matrix-matched ones, within a sequence of mixed commodities)

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Supplement B (informative):

Validation Data

Validation data from interlaboratory validation studies round 1 to 3 is available in tabular form as Excel-Sheet here: <u>QuPPe.eu - EN Supplements</u>

Supplement C (informative):

Additional information

Table SC.1 — Conversion factors between typical purchased standards and target analytes

Compound	MW [g/mol]	Compound as sold	MW [g/mol]	Conversion Factor (CF)	Inverse CF
Bromide (anion)	79,9	Potassium bromide	119,0	0,67	1,49
Chlorate (anion)	83,5	Chlorate-sodium	106,4	0,78	1,27
Chlormequat (cation) ^a	122,6	Chlormequat-chloride ^a	158,1	0,78	1,29
Chlormequat-D ₄ (cation)	126,6	Chlormequat-D ₄ -chloride	162,1	0,78	1,28
Difenzoquat (cation)	249,3	Difenzoquat-methylsulfate	360,4	0,69	1,45
Difluoroacetic acid-13C2	96,0	Sodium difluoroacetate-13C2	120,0	0,80	1,25
Fosetyl	110,0	Fosetyl-Al	118,0 ^b	0,93	1,07
Fosetyl-D ₅	115,0	Fosetyl-D ₅ -Al	123,0b	0,93	1,07
		Fosetyl-D ₅ -sodium	137,0	0,84	1,19
Glufosinate	181,1	Glufosinate-ammonium	198,2	0,91	1,09
Glufosinate-D ₃	184,1	Glufosinate-D ₃ -ammonium hydrate	243,2	0,76	1,32
		Glufosinate-D ₃ -hydrochloride	220,6	0,83	1,20
Mepiquat (cation) ^a	114,2	Mepiquat-chloride ^a	149,7	0,76	1,31
Mepiquat-D ₃ (cation)	117,2	Mepiquat-D ₃ -iodide	244,1	0,48	2,08
Mepiquat-4-hydroxy	130,2	Mepiquat-4-hydroxy-chloride	165,7	0,79	1,27
N-Acetyl-Glufosinate	223,2	N-Acetyl-Glufosinate-disodium	267,2	0,84	1,20
N-Acetyl-Glufosinate-D ₃	226,2	N-Acetyl-Glufosinate-D3-disodium	270,2	0,84	1,19
Nereistoxin	149,3	Nereistoxin-oxalate	239,3	0,62	1,60
Nereistoxin-D ₆	155,3	Nereistoxin-D ₆ -oxalate	245,3	0,63	1,58
Nicotine	162,2	Nicotine hemisulfate	422,5 ^c	0,77	1,30
Propamocarb-N-oxide	204,3	Propamocarb-N-oxide hydrochloride	240,7	0,85	1,17
Trifluoroacteic acid	113,0	Sodium trifluoroacetate	136,0	0,83	1,20
Trifluoroacteic acid-13C2	115,0	Sodium trifluoroacetate-13C2	138,0	0,83	1,20
Trimethylsulfonium (cation)	77,2	Trimethylsulfonium-iodide	204,1	0,38	2,64
Trimethylsulfonium-D ₉ (cation)	86,2	Trimethylsulfonium-D9-iodide	213,1	0,40	2,47

^a Attention: The current EU – MRLs are expressed as the respective chloride salts. Thus, no mathematical conversion of the chloride to the cation is needed.

b The MW given here includes only 1/3 of the aluminium mass (one aluminium ion is bound to three fosetyl anions)

c The MW refers to the following formula $(C_{10}H_{14}N_2)_2 \cdot H_2SO_4$ which entails two nicotine molecules.

 ${\it Table SC.2-Exemplary\ concentrations\ and\ solvents\ of\ stock\ and\ working\ solutions\ of\ analytes\ and\ their\ respective\ IL-ISs}$

Compound	Stock Solution (exemplary)		Working Solutions including mixtures (exemplary)		
	Solvent used to prepare	[mg/mL]	Solvent used to prepare	[μg/mL]	
Aminocyclopyrachlor	МеОН	1	МеОН	10 / 1 / 0,1	
Amitrole	МеОН	1	МеОН	10 / 1 / 0,1	
AMPA	10 % ACN in water	1	10 % ACN in water	10 / 1 / 0,1	
Bromide	МеОН	1	МеОН	10 / 1 / 0,1 / 0,01	
Chlorate	МеОН	1	МеОН	10 / 1 / 0,1 / 0,01	
Chloridazon-desphenyl	МеОН	1	МеОН	10 / 1 / 0,1	
Chlormequat	МеОН	1	МеОН	10 / 1 / 0,1	
Cyanuric acid	МеОН	1	10 % ACN in water	10 / 1 / 0,1	
Cyromazine	МеОН	1	МеОН	10 / 1 / 0,1	
Daminozide	МеОН	1	МеОН	10 / 1 / 0,1	
Difenzoquat	ACN	1	МеОН	10 / 1 / 0,1	
Difluoroacetic acid	ACN with 5% water	1	ACN with 5% water	10 / 1/ 0,1	
Ethephon	10 % ACN in water + 0,1 % HCl	1	10 % ACN in water + 0,1 % HCl	10 / 1 / 0,1	
ETU	МеОН	1	МеОН	10 / 1 / 0,1	
Fosetyl	10 % ACN in water	0,1	10 % ACN in water	10 / 1 / 0,1	
Glufosinate	10 % ACN in water	1	10 % ACN in water	10 / 1 / 0,1	
Glyphosate ^b	10 % ACN in water	1	10 % ACN in water	10 / 1 / 0,1	
НЕРА	10 % ACN in water	1	10 % ACN in water	10 / 1 / 0,1	
Matrine	ACN	1	ACN	10 / 1 / 0,1	
Maleic Hydrazide	МеОН	1	10 % ACN in water	10 / 1 / 0,1	
Melamine	MeOH:water (90:10)	1	МеОН	10 / 1 / 0,1	
Mepiquat	МеОН	1	МеОН	10 / 1 / 0,1	
Mepiquat-4-hydroxy	МеОН	1	МеОН	10 / 1 / 0,1	
MPPA	10 % ACN in water	1	10 % ACN in water	10 / 1 / 0,1	
N-Acetyl-Glufosinate	10 % ACN in water	1	10 % ACN in water	10 / 1 / 0,1	
N-Acetyl-Glyphosate	10 % ACN in water	1	10 % ACN in water	10 / 1 / 0,1	
Nereistoxin	MeOH / water (3:1)	1	МеОН	10 / 1 / 0,1	
Nicotine ^b	ACN	1	ACN	1 / 0,1	
Oxymatrine	ACN	1	ACN	10 / 1 / 0,1	

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Compound	Stock Solution (exemplary)	Working Solutions including mixtures (exemplary)		
	Solvent used to prepare	[mg/mL]	Solvent used to prepare	[µg/mL]
Perchlorate	МеОН	1	МеОН	10 / 1 / 0,1 / 0,01
Phosphonic acid ^b	Water (180-H ₂ O for the ILIS)	1	ACN ^c	10 / 1 / 0,1 / 0,01
Propamocarb	ACN	1	МеОН	10 / 1 / 0,1
Propamocarb-N- desmethyl	ACN:Acetone (1 mL acetone to initially dissolve)	1	МеОН	10 / 1 / 0,1
Propamocarb-N-oxide	МеОН	1	МеОН	10 / 1 / 0,1
PTU	МеОН	1	МеОН	10 / 1 / 0,1
Triazole-lactic acid	МеОН	1	МеОН	10 / 1 / 0,1
Triazole-acetic acid	МеОН	1	МеОН	10 / 1 / 0,1
Triazole-alanine	MeOH / water (1:3)	1	МеОН	10 / 1 / 0,1
Trifluoroacetic acid	ACN with 5% water	1	ACN with 5% water	10 / 1/ 0,1
Trimethylsulfonium	МеОН	1	MeOH	10 / 1 / 0,1

^a Use plastic vials and protect solutions from light exposure.

MeOH: Methanol; ACN: Acetonitrile; FA: Formic acid.

b Use plastic vessels and stoppers for compounds that tend to interact with glass surfaces.

 $^{^{\}text{C}}\,$ Pure water (18O-H2O for the IL-IS) is also suitable for the working solution. 10% ACN will reduce growth of microorganisms.